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Effect of heavy metals and other xenobiotics on biodegradation of waste canola oil by cold-adapted *Rhodococcus* sp. strain AQ5-07

Efecto de metales pesados y otros xenobioticos en la biodegradación de residuos de aceite de canola mediante la cepa adaptada al frío *Rhodococcus* sp. AQ5-07

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Abstract

The Antarctic is generally considered to be one of the most pristine areas in the world. However, both long and short-range pollutants are now known to be present in the Antarctic environment. Canola oil is an example of a polluting hydrocarbon that can be accidentally released into the Antarctic environment in oil wastewater treatment plants. The Antarctic soil bacterial strain *Rhodococcus* sp. AQ5-07, known to be capable of using waste canola oil (WCO) as its sole source of carbon, was tested for its ability to degrade canola oil in the presence of different heavy metals and xenobiotics. *Rhodococcus* sp. AQ5-07 was grown on minimum salt media containing different heavy metals (Zn, Co, Ni, Ag, Pb, Cu, Cr, Hg, Cd and As), xenobiotics (acrylamide and phenol) supplemented with 3% WCO. Three out of the 10 heavy metals tested (Hg, Cd and Ag) led a significant reduction in canola oil degradation at a concentration of 1 ppm. The IC_{50} values of Hg, Cd and Ag were 0.38, 0.45 and 0.32 ppm, respectively. The strain could also withstand 10 mg/L acrylamide, 50 mg/L phenol and 0.5% (v/v) diesel. This study confirmed the ability of *Rhodococcus* sp. AQ5-07 to degrade canola oil in the presence of various heavy metals and other xenobiotics, supporting its potential use in bioremediation of vegetable oil and wastewater treatments in low temperature environments.
Keywords: Arctica, biodegradation, canola oil, heavy metals, *Rhodococcus*, xenobiotics.

Resumen

La Antártida se considera generalmente una de las áreas más prístinas del planeta. Sin embargo, es sabido que contaminantes de largo y corto alcance están presentes en el medio ambiente antártico. El aceite de canola es un ejemplo de un hidrocarburo contaminante que puede ser liberado accidentalmente al medio ambiente antártico por las plantas de tratamiento de aguas residuales. La cepa bacteriana de suelo antártico *Rhodococcus* sp. AQ5-07, de la cual se sabe que es capaz de usar aceite de canola de desecho (ACD) como única fuente de carbono, fue testeada en su capacidad para degradar el aceite de canola en presencia de diferentes metales pesados y xenobióticos. *Rhodococcus* sp. AQ5-07 se cultivó en medio de cultivo mínimo de sales conteniendo diferentes metales pesados (Zn, Co, Ni, Ag, Pb, Cu, Cr, Hg, Cd y As), xenobióticos (acrilamida y fenol) suplementado con 3% de ACD. Tres de los 10 metales pesados probados (Hg, Cd y Ag) produjeron una reducción significativa en la degradación del aceite de canola a una concentración de 1 ppm. Los valores de CI50 de Hg, Cd y Ag fueron 0,38, 0,45 y 0,32 ppm, respectivamente. La cepa también pudo soportar 10 mg/L de acrilamida, 50 mg/L de fenol y 0.5% (v/v) de diesel. Este estudio confirmó la capacidad de *Rhodococcus* sp. AQ5-07 para degradar el aceite de canola en presencia de varios metales pesados y otros xenobióticos, apoyando su potencial uso en la biorremediación de aceites vegetales y tratamientos de aguas residuales en ambientes de baja temperatura.
Palabras clave: Antártica, biodegradación, aceite de canola, metals pesados, *Rhodococcus*, xenobióticos.

1   Introduction

Antarctica is a unique region globally, and a key natural laboratory devoted to scientific research. It is often assumed to be one of the most pristine regions in the world (Xie and Sun, 2008), but it is not sealed from the rest of the world and is reached by global contaminants (Bargagli, 2005). Since the early 20th century, coastal regions have suffered the direct impacts of human presence (Vodopivez *et al.*, 2015). Anthropogenic pollution can reach Antarctica areas through atmospheric circulation and deposition. Over the last 200 years, human activities in Antarctica have changed focus from initial exploration towards scientific research and, more recently, tourism. Since the International Geophysical Year of 1957/1958, scientific research programmes have developed to a level where now approximately 5000 researchers and support staff work in Antarctica annually (Tiwari, 2017; COMNAP, 2012). A further surge in activity took place with the International Polar Year of 2007/2008 (Convey *et al.*, 2012). At the same time, by the 2015/16 austral summer, tourist numbers increased to almost 40,000 (IAATO, 2017), a trend that is expected to continue. Today, nationals of more than 30 countries are actively involved in scientific research on the continent (Williams *et al.*, 2017; Rack, 2015). The increasing levels of human activity in the region inevitably are associated with risks of anthropogenic pollution events that have become a significant threat to the natural Antarctic environment.

Heavy metals occur naturally in the Earth's crust. They can represent a serious environmental threat due to accumulation, as they cannot be broken down like other contaminant types (Buendía-González *et al.*, 2019; Corral-Escárceg *et al.*, 2017; Chu *et al.*, 2019). Elements such as copper, lead and arsenic are often present in limited quantities but the threat also extends to other elements, metalloids and metallic elements such as Sb, Tl, Mo, Rh, Ir, Pt, U, Bi, Ba, Cd, Ag, Co, Zn, Mn, Cr and V (Hong *et al.*, 2012; Soyol-Erdene *et al.*, 2011). The presence of heavy metals in contaminated areas can therefore be a major challenge for bioremediation of other pollutants such as oils and other hydrocarbons (Alcázar-Medina *et al.*, 2020; Hernández-Martínez *et al.*, 2019; Interiano-López *et al.*, 2019; Suárez-García *et al.*, 2019; Subramaniam *et al.*, 2019; Karamba *et al.*, 2016; Ruiz-Marín *et al.*, 2013).

Remediation of lipid-contaminated areas through biodegradation provides an important example of recovery from pollution. Canola oil, a vegetable oil, is a major cooking oil widely used in food preparation both industrially and at domestic scale. Fats, fatty acids (Fas), oil, and grease are released into the natural environment together with industrial and municipal wastewater (Čipinyte *et al.*, 2009; Ibrahim *et al.*, 2018). These polluting compounds can lead to serious anthropogenic problems such as odour, pipe and filter blockage, oil film formation and oxygen depletion, with deleterious effects on aquatic organisms and ecosystems (Fadile *et al.*, 2011). Metabolism of lipids involves emulsification and degradation. Free fatty acid(s) and glycerol are generated during the degradation process. The free fatty acid(s) are converted to acetyl-CoA via the beta-oxidation pathway and finally citric acid cycle (Phong *et al.*, 2014), while the glycerol generated is converted to glucose via a series of chemical reactions.

Many aerobic and anaerobic microorganisms capable of degrading lipids have been isolated from water and soil samples (Ibrahim *et al.*, 2018). These are commonly bacterial and fungal strains from the genera *Rhodococcus*, *Staphylococcus, Arthrobacter, Pseudomonas, Enterobacter, Bacillus, Penicllium, Serratia, Aspergillus, Mucor, Burkholderia, Acinetobacteria* and *Lactobacillus* (Danikuu and Sowley, 2014; Nagarajan *et al.*, 2014; Phong *et al.*, 2014; Bharathi *et al.*, 2012; Kumar *et al.*, 2012; Čipinyte *et al.*, 2009; Matsumiya *et al.*, 2007). These organisms have the capacity to release extracellular lipases that are responsible for vegetable oil degradation (Serikovna *et al.*, 2013; Pereira *et al.*, 2003).

Considerable attention has been given in microbial biotechnology to the development of enzymatic lipases (Dors *et al.*, 2013). Several studies have shown that lipolytic aerobic treatment techniques do not develop odour problems and lead to better effluent quality (Chan *et al.*, 2009). The use of microbial enzymes in lipid degradation methodologies is considered to improve efficiency, as they are eco-friendly, specific, reduce time investment, are easy to use, disease-free and cost-effective, as well as reducing chemical oxygen demand as well as the quantity of suspended lipid solids and colour (Dors *et al.*, 2013).

The previously isolated strain *Rhodococcus* sp. AQ5-07, a known phenol-degrading and cold-adapted Antarctic soil bacterium (Lee *et al.*, 2018), was tested here for its ability to degrade waste canola oil (WCO) as a sole source of carbon in the presence of different

types of heavy metal and other xenobiotic compounds. To our knowledge, no studies to date have examined the effects of heavy metals and other xenobiotics on the biodegradation activity of WCO using cold-adapted bacteria.

2 Materials and methods

2.1 Chemicals

Waste canola oil was collected from the Chilean Bernardo O'Higgins Riquelme Station, northern Antarctic Peninsula, in February 2018. Analytical grade chemicals were obtained from Fisher Scientific (Malaysia), Sigma (USA) and Merck (Darmstadt, Germany).

2.2 Microorganism culture and media preparation

Previously isolated *Rhodococcus* sp. strain AQ5-07 originally obtained from King George Island (South Shetland Islands, Antarctica) was cultured at 10°C in sterilised canola oil liquid medium. The liquid medium is used to enrich the lipid-degrading organism contained 3% canola oil and the following compositions (mg/L): 100 $(NH_4)_2SO_4$, 30 yeast extract, 20 $MgSO_4$, 60 KH_2PO_4, 90 K_2HPO_4. For solid medium, Tween-peptone agar was prepared containing the following (mg/L): 10 $CaCl_2$, 50 NaCl, 100 peptone, 180 agar and 5 mL/L Tween 80 (Aktas *et al.*, 2002). The media were autoclaved for 20 min at 121°C. The isolate was maintained and sub-cultured every two weeks in Tween-peptone agar medium.

2.3 Flask culture experiments

A single loop of a freshly grown culture from a Tween-peptone agar plate was transferred to 10 mL nutrient broth medium and incubated on a rotary shaker at 150 rpm and 10°C for 24 hr. Ten percent (v/v) of the culture was transferred to 100 mL of the canola oil medium in 250 mL Erlenmeyer flasks in triplicates and incubated for 72 hr at 150 rpm and 10°C. After 72 hr, the bacterial growth was measured according to colony forming units (CFU/mL) procedure of Hazan *et al.* (2012). Dilutions of 10-1, 10-2, 10-3, 10-4, 10-5, 10-6 and 10-7 made with 900 μL normal saline were carried out. Then, 100 μL of the suspension from 10-2 to 10-7 dilutions were aseptically transferred to petri

dishes followed by spreading the bacterial suspension to allow proper mix between the agar and bacterial suspension. The plates were then incubated at 15°C for 48 hr.

After the incubation, petri dishes containing between 30 and 300 colonies were collected and counted. Colony that forms the units per millilitre were calculated by multiplying the number of colonies by the dilution factor and divide by the volume of bacterial suspension plated on the agar (Equation 1) (Sieuwerts *et al.*, 2008).

$$CFU/mL = \frac{No\ of\ colonies\ counted}{Volume\ of\ sample\ plated} \times dilution\ factor \tag{1}$$

Control tubes containing the media and oil without the bacterial inoculation were used to ensure the dependency of observed growth on the inoculated bacteria.

2.4 Determination of canola oil degradation by gravimetric method

The amount of residual oil was determined using a gravimetric method following Abubakar *et al.* (2019) and Sihag and Pathak (2016). Fresh bacterial culture (OD600 1.3 - 1.4) was inoculated into 100 mL MSM media supplemented with 3% WCO. After 72 hr incubation, 1 mL was removed and bacterial growth was measured by assessment of colony forming units (CFU/mL).

For extracting the residual oil, 10 mL of n-hexane was added to the medium and the mixture was separated using a separating funnel. Two layers were formed; the lower layer that included the medium and the upper layer including the oil. The oil from the upper layer was collected in a pre-weighed Petri dish. One millilitre of filtered residual oil was used for GC-MS analysis. The percentage of canola oil degradation was calculated using Equation 2.

$$Canola\ oil(\%) = \frac{(a-b)}{a} \times 100\% \tag{2}$$

where a is the mass of canola oil added to the medium and b is the mass of residual canola oil.

2.5 Screening for heavy metal inhibition

A batch of experiment was conducted in a conical flask with initial WCO concentration of 3% (v/v) and the strain AQ5-07 was cultivated in nutrient broth (NB). About 10% (v/v) of the seed cultures were transferred

to 100 mL of WCO liquid media. Assessment of bacterial growth and oil degradation was carried out at every 12 hr intervals. The effect of different heavy metals on WCO degradation was studied. Heavy metals ions, namely zinc (Zn), cobalt (Co), nickel (Ni), silver (Ag), lead (Pb), copper (Cu), chromium (Cr), mercury (Hg), cadmium (Cd) or arsenic (As) at a concentration of 1 ppm, were added into the canola oil media. The media was incubated in an optimised condition for of $(NH_4)_2SO_4$ 1.0 g/L, yeast extract 0.3 g/L, temperature of 10°C, pH 7.5 and substrate concentration of 3% v/v. The amount of bacterial growth and degradation were then measured. Heavy metal ions that inhibited bacterial growth and oil-degrading ability were selected for further studies. All the experiments were conducted in triplicate and the results obtained for WCO were compared to confirm whether the metals caused a reduction in oil degradation. Media without the addition of heavy metals served as controls.

2.6 Effects of xenobiotics

The effect of xenobiotics (acrylamide, phenol and diesel) on WCO degradation was studied. Different concentrations were selected based on the literature (Lee *et al*., 2018; Rajasekar *et al*., 2007), and the media were incubated under optimised conditions for 72 hr at 10°C on a rotary shaker at 150 rpm.

2.7 Standardisation of parameters

The concentration of the selected heavy metals was manipulated, ranging between 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 ppm, while keeping all other parameters constant. Aliquots of *Rhodococcus* sp. AQ5-07 (10%) were transferred to 100 mL of each canola oil medium flask containing different concentrations of the heavy metals.

2.8 Inhibitory concentration 50 (IC_{50})

The inhibitory concentration (IC_{50}) was defined as the concentration that inhibited oil degradation by 50%. This test is used to determine best fit for a particular application from a list of various heavy metals concentrations. Tubes were set sequentially in decreasing order of concentration and incubated for 72 hr and the IC_{50} was calculated. To determine the IC_{50} values, the concentration range used of each heavy metals were from 0 - 1.0 ppm, and the data were analysed using Graphpad Prism v 8

software, by plotting % degradation against heavy metals concentration in ppm. All the analyses were conducted in triplicate and the mean ± standard deviation values were taken.

2.9 Statistical analyses

Analysis of variance, using SPSS statistics V. 24 software package (SPSS Inc., Chicago, Illinois, USA), was used to compare results within treatment groups, and Tukey's test was used to conduct *post hoc* pairwise tests if significant differences were obtained. All experiments were carried out in triplicate and data are presented as a mean ± standard deviation.

3 Results and discussion

3.1 Canola oil degradation

Cold-adapted *Rhodococcus* sp. AQ5-07 was previously tested in canola oil media for its ability to degrade WCO in the absence of heavy metals (Ibrahim *et al*. in review). In this study, 3% WCO was used as it is the optimum initial oil concentration exhibited by the strain (Fig. 1). Canola oil, as with vegetable oil generally at high concentrations (Shon *et al*., 2002), is known to be toxic to microorganisms, and efficient degradation demands the selection of a threshold level of canola oil that can be tolerated and degraded efficiently.
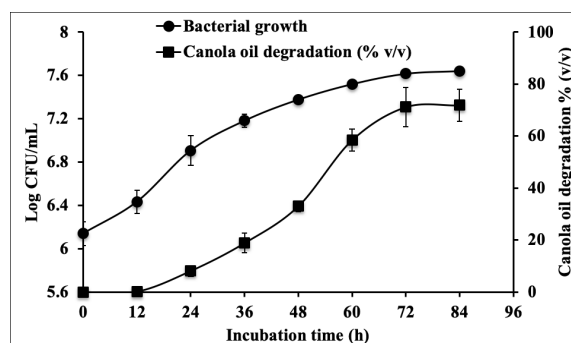

Fig. 1. Degradation and bacterial growth with a 3% initial canola oil concentration by *Rhodococcus* sp. AQ5-07 over 72 hr incubation. *Error bars* represent mean ± standard deviation, *n* = 3.
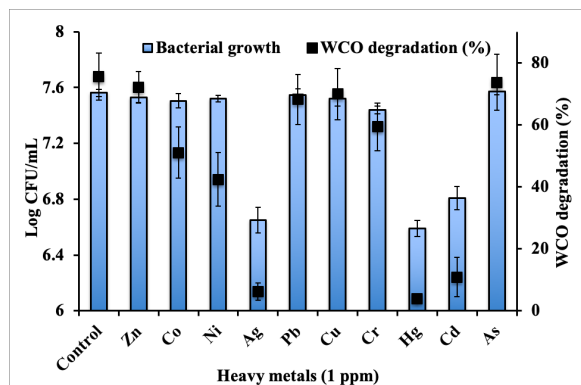
Fig. 2. Effect of 1 ppm heavy metal ion concentrations on canola oil degradation and bacterial growth of *Rhodococcus* sp. AQ5-07 after 72 hr incubation. Error bars represent mean ± standard deviation, $n = 3$.

3.2 Effects of heavy metal on canola oil degradation

The effects of different heavy metals on WCO degradation by *Rhodococcus* sp. AQ5-07 were compared after 72 h incubation. Three of the 10 heavy metals tested had an inhibitory effect on WCO degradation (Fig. 2), Ag, Hg, and Cd. Hg had the strongest inhibition effect with only 3.88% of WCO being degraded, followed by Ag (6.15%) and Cd (10.90%). One-way ANOVA confirmed significant differences between the different heavy metals tested in terms of bacterial growth and oil degradation (F (10, 22) = 176.51, $p < 0.001$ and F (10, 22) = 48.61, $p < 0.001$, respectively). *Post hoc* tests identified no significant differences in degradation between control and Zn, Pb, Cu, Cr and As (all $p > 0.05$), while the remaining heavy metals showed significant differences with the control (all $p < 0.01$). None of the metals tested enhanced the degradation rate, and control medium without the addition of any heavy metals showed the highest rate.

Ahmad *et al*. (2018) and Zakaria *et al*. (2018) reported similar findings in studies of Antarctic Arthrobactar bambusae AQ5-003 and *Rhodococcus baikonurensis* AQ5-001, with Hg, Ag and Cd again inhibiting bacterial growth and phenol degradation. Abou-Shanab *et al*. (2007) reported that Zn, Ni, Cr and Hg inhibited Arthrobacter sp. AY509239. More widely, the detrimental effect of heavy metals on aquatic vertebrates include increased respiration, impaired fertilisation and reduced survival (Stebbing, 2002).
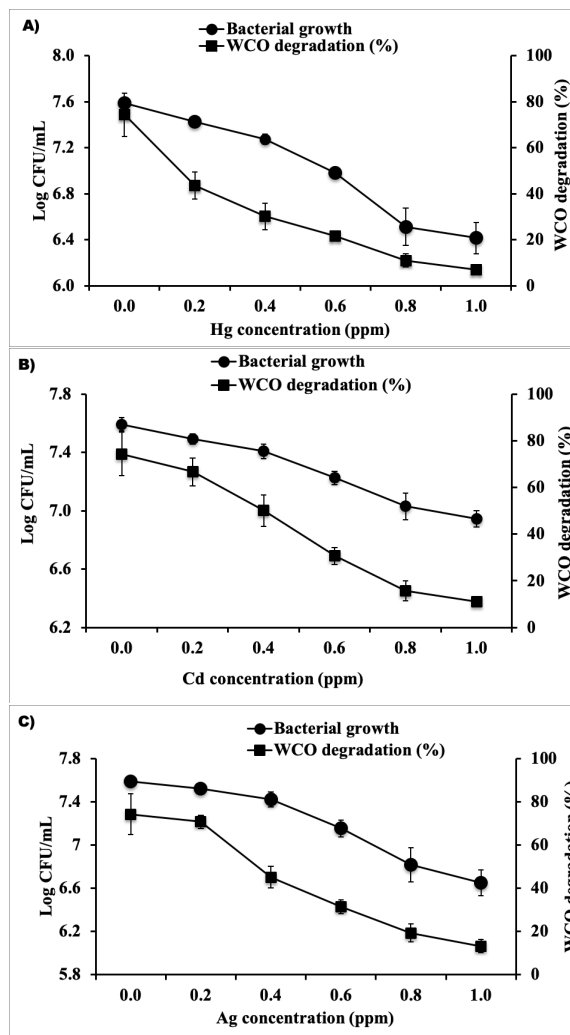

Fig. 3. Effect of a) Hg b) Cd and c) Ag concentration on canola oil degradation and growth of Rhodococcus sp. AQ5-07 after 72 hr incubation. Error bars represent mean ± standard deviation, $n = 3$.

The pattern of microbial growth inhibition by heavy metals is often specific for each organism and each metal and, for any given metal, the toxic concentration may vary between different organisms (Sadler and Trudinger, 1967). Rajendran *et al*. (2003) reported that many heavy metals could affect microorganisms even at low concentration. As with most antimicrobial substances, the effects of heavy metals depend on their concentration which may be either bacteriostatic, where growth is inhibited but the microorganisms are not destroyed, or bactericidal, where the microorganisms are destroyed and will not recover even if the inhibitors are removed. It has been reported that both biochemical and

morphological changes in microorganisms can be observed at sub-lethal heavy metal concentrations (Sadler and Trudinger, 1967). Biochemical changes include changes in the proportion of some molecular components, particularly nucleic acids, which are stimulated in some organisms by cobalt, while the activities of certain oxidative enzymes are decreased (Nies, 1999). Morphological changes include the abnormal production of long filamentous forms when exposed to platinum and the conversion of bacterial rods to spherical forms. These may result in the inhibition of normal cell division and cell wall synthesis processes (Sharma *et al.*, 2014).

The effect of Hg on WCO degradation was studied in more detail at concentrations between 0 and 1 ppm. Hg concentrations from 0.2 to 1 ppm reduced canola oil-degrading ability (Fig. 3a). Increasing Hg concentration above 0.4 ppm led to a significant decrease in canola oil degradation of 30.1%. One-way ANOVA revealed an overall significant difference between different Hg concentrations in both bacterial growth and oil degradation ($F_{(5, 12)} = 79.95$, $p < 0.001$ and $F_{(5, 12)} = 64.57$, $p < 0.001$, respectively). *Post hoc* comparisons indicated that there were no significant differences in terms of bacterial growth between the control (M = 7.59, SD = 0.04), and 0.2 ppm (M = 7.42, SD = 0.05) and 0.4 ppm (M = 7.27, SD = 0.04). In terms of oil degradation, there were significant differences between control and all Hg concentrations (all $p < 0.001$). Hg is the most toxic non-radioactive metal in the natural environment and is toxic in any form (Abdel-Salam *et al.*, 2010; Ross, 2004). Hg exerts its inhibitory effects by binding to sulfhydryl, phosphoryl, amide, carboxylic and amine groups of proteins. Robinson and Tuovinen (1984) reported that Hg is soluble in lipids and can easily bind to proteins containing sulfhydryl groups in the cell membrane, as well as to enzymes, causing toxicity. Mercury toxicity to bioremediation activity can be reduced by the addition of metal immobilising agents (Yusuf *et al.*, 2019). Ibrahim *et al.* (2016) reported that immobilised *Leifsonia* sp. strain SIU can withstand up to 0.4 ppm Hg in caffeine degradation, helping to reduce Hg toxicity and maintaining the function of the bioremediation agent even in a polluted environment.

The effects of cadmium concentration on WCO degradation were studied at concentrations between 0 and 1 ppm. Cd concentrations from 0.4 to 1 ppm reduced canola oil-degrading ability (Fig. 3b). One-way ANOVA again identified overall significant differences in bacterial growth and oil degradation between the different concentrations ($F_{(5, 12)} = $ 62.84, $p < 0.001$ and $F_{(5, 12)} = 62.00$, $p < 0.001$, respectively). *Post hoc* comparisons indicated that there were no significant differences (p = 0.595) between the degradation achieved between the control (M = 74.34, SD = 9.42) and 0.2 ppm concentration (M = 66.60, SD = 5.97), but there were significant differences between the control and all other Cd concentrations (all $p < 0.001$). Cadmium occurs naturally in the soil environment but, due to human activities, it is more widely present. Ahmad *et al.* (2018) reported that cadmium inhibited phenol degradation by Antarctic *Arthrobacter bambusae* strain AQ5-003, being toxic even at 0.1 ppm. In contrast, Cd can also enhance bacterial growth and degradation in another Antarctic bacterium, *Rhodococcus baikonurensis* strain AQ5-001 (Zakaria *et al.*, 2018). Cd also inhibits the biodegradation of other xenobiotics (Hoffman *et al.*, 2005; Sandrin and Maier, 2003). The inhibitory effect of Cd can be lessened by immobilising the bacteria (Ahmad *et al.*, 2017; Ibrahim *et al.*, 2016). Furthermore, the addition of chemical additives can reduce the toxicity of cadmium ions (El-Deeb and Altalhi, 2009). Finally, the effects of various Ag concentrations on WCO degradation were studied from 0 to 1 ppm. The results obtained demonstrated that concentrations from 0.6 to 1 ppm reduced canola oil-degrading ability by 31.49, 19.23 and 13.14%, respectively (Fig. 3c). A significance difference was observed between different Ag concentrations in both analyses as shown by analysis of variance ($F_{(5, 12)} = 51.14$, $p < 0.001$ and $F_{(5, 12)} = 75.18$, $p < 0.001$, respectively). In *post hoc* tests the degradation achieved under control conditions (M = 74.34, SD = 9.42) and 0.2 ppm concentration (M = 70.77, SD = 3.09) did not differ significantly, but there were significant differences between the control and all other Ag concentrations (all $p < 0.001$). The strain AQ5-07 degraded 13.14% WCO in the presence of 1.0 ppm Ag, thus confirming silver as a strong inhibitor of canola oil degradation.

Silver can be activated in the presence of cytochrome b and exerts its toxicity to bacteria by penetration of the silver ions through the plasma membrane and the bacterial cell wall, thus inactivating membrane-bound proteins. The ion also binds to bacterial DNA, interrupting replication. Silver ions can also damage the ability of ribosomes to translate mRNA into proteins (Chudobova *et al.*, 2013). Nies (1999) reported that Ag in coins and medicine can act as an anti-microbial agent.

3.3 Inhibitory concentration 50 (IC_{50})

The half maximal inhibitory concentration (IC_{50}) is an effective measurement of the activity of a substance in preventing a particular biochemical or biological function. In the current study, the IC_{50} is the concentration that reduces canola oil degradation by 50%. The IC_{50} values of Hg, Cd and Ag for canola oil degradation by *Rhodococcus* sp. AQ5-07 are shown in Fig. 4a, b and c. The IC_{50} values were 0.3866 ppm (Hg), 0.4539 ppm (Cd) and 0.3217 ppm (Ag). The IC_{50} value for Hg inhibition by Antarctic *Rhodococcus baikonurensis* strain AQ5-001 was 0.5 ppm (Zakaria *et al.*, 2018). Ahmad *et al.* (2018) reported IC_{50} values for *Arthrobacter bambusae* strain AQ5-003 of 0.04 ppm for Cd and 0.53 ppm for Ag and concluded that Cd was the most toxic metal of those tested.
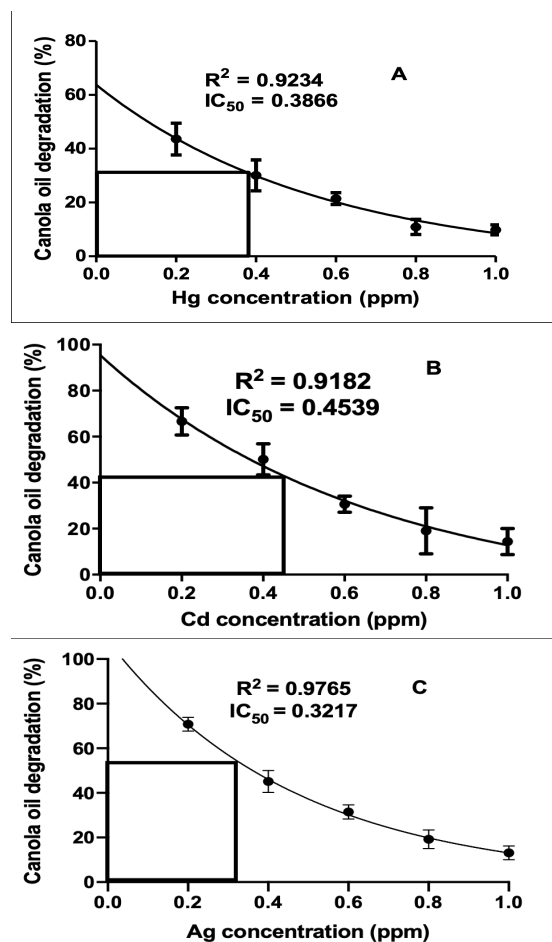

Fig. 4. The IC_{50} of a) Hg b) Cd and c) Ag on canola oil degradation by *Rhodococcus* sp AQ5-07. *Error bars* represent mean ± standard deviation, *n* = 3.

3.4 Effects of xenobiotics (acrylamide, phenol and diesel) on canola oil degradation

The effects of different acrylamide concentrations on WCO degradation by *Rhodococcus* sp. AQ5-07 were studied at concentrations from 0 - 200 mg/L over 72 hr incubation. Acrylamide concentrations from 10 - 200 mg/L reduced canola oil degradation (Fig. 5a). One-way ANOVA showed overall significant differences in oil degradation and bacterial growth between different acrylamide concentrations (F (5, 12) = 28.63, *p* < 0.001 and F (5, 12) = 69.09, *p* < 0.001, respectively).

There were no significant differences between the degradation achieved by the control (M = 68.50, SD = 7.90) and 10.00 mg/L (M = 60.30, SD = 6.81), but there were significant differences between the control and all other acrylamide concentrations (all *p* < 0.001). At 200 mg/L, there was a significant decrease in oil degradation (19.20%), which may be due to the inhibitory effect of acrylamide on thiol groups of proteins (Buranasilp and Charoenpanich, 2011). These data suggest that, at high concentrations, acrylamide is toxic to this bacterial strain. Shukor *et al.* (2009a) similarly reported a decrease in bacterial growth at high acrylamide concentration above 500 mg/L in Antarctic *Pseudomonas* sp. strain DRYJ7. Nawaz *et al.* (1998) and Buranasilp and Charoenpanich (2011) compared acrylamide degradation by free and immobilised cells of *Rhodococcus* sp. and *Enterobacter aerogenes*, respectively, and found that immobilised cells degraded acrylamide to acrylic acid and ammonia faster than free cells.

The effects of different phenol concentrations on WCO degradation by *Rhodococcus* sp. AQ5-07 were studied from 0 - 500 mg/L over a 72 hr incubation. Phenol concentrations from 50 - 500 mg/L affected canola oil degradation (Fig. 5b). One-way ANOVA confirmed that the effects of different phenol concentrations on bacterial growth and oil degradation were significant (F (5, 12) = 25.77, *p* < 0.001 and F (5, 12) = 20.77, *p* < 0.001, respectively). *Post hoc* tests indicated that there were no significant differences in the growth and degradation achieved in the control (M = 68.50, SD = 7.90), 10 mg/L (M = 70.44, SD = 3.90) and 50 mg/L (M = 51.02, SD = 8.30) phenol concentrations, while the remaining pairwise comparisons with the control were significantly different. At 10 mg/L, there was a slight increase in WCO degradation, which is due

to the effect of the phenol. At 500 mg/L, there was a significant decrease in oil degradation (27.40%), which may be due to the toxic effect of phenol on the bacterium. According to Wang *et al*. (2007) maximum phenol degradation by Acinetobacter sp. strain PD 12 was achieved at 500 mg/L. It has also been reported that, at high phenol concentrations, the compound inhibits bacterial growth. Lee *et al*. (2018) recorded maximum phenol degradation at 500 mg/L by the Antarctic strains Arthrobacter sp. AQ5-05 and AQ5-06 and *Rhodococcus* sp. AQ5-07.

The effects of different initial concentrations of diesel on WCO degradation by *Rhodococcus* sp. AQ5-07 were studied from 0 - 2.5% after 72 hr incubation. Diesel concentrations from 1 - 2.5% reduced canola oil degradation (Fig. 5c). This suggests that the optimum carbon source (WCO and diesel) initial concentrations for the growth of strain AQ5-07 were 3% (v/v) and 0.5% (v/v) diesel, respectively. One-way ANOVA identified overall significant differences in bacterial growth and oil degradation between different diesel concentrations ($F_{(5, 12)} = 30.04$, $p < 0.001$ and $F_{(5, 12)} = 31.84$, $p < 0.001$, respectively). *Post hoc* comparisons showed that there were significant differences between degradation achieved between the control and all diesel concentrations (all $p < 0.001$).

Canola oil in the presence of diesel is required as a carbon source at certain concentrations. According to Shukor *et al*. (2009b), diesel can be toxic to microorganisms due to the solvent effect of diesel, which destroys the bacterial cell membrane. Therefore, many studies on microbial degradation of diesel have been carried out using lower diesel concentrations ranging from 0.5 - 1.5% (v/v) (Rajasekar *et al*., 2007). This result is consistent with that reported by Shukor *et al*. (2009b) which found that, at high diesel concentrations of 1 or 1.5% (v/v), degradation is generally slow and toxic to bacteria. Diesel degradation at higher concentrations greater than 5.5% (v/v) has been reported, but requires additional yeast extract (1 g/L) and glucose (2 g/L) (Kwapisz *et al*., 2008). Since *Rhodococcus* sp. AQ5-07 was able to withstand a high concentration of diesel, this implies that the strain is a good candidate for canola oil bioremediation when diesel is present as a co-contaminant.

Due to increased in human activities like tourism and scientific research have led to the risk of diesel pollution. The spilled diesel may serve as a substrate for microbial growth. Oil and other xenobiotics pollutants in Antarctica tends to be persistent in the region due to extremely low temperatures and often dryness limit the rates of bioprocesses in addition to abiotic degradation (Lee *et al*., 2018).

Conclusions

The present study revealed the effect of heavy metals and other xenobiotics on canola oil biodegradation using a recently isolated cold-adapted Antarctic bacterium, Rhodococcus sp. AQ5-07. Three of the ten heavy metals tested, namely Ag, Cd and Hg, had a strongly inhibitory effect on bacterial growth and oil degradation at concentrations of 1 ppm. Hg and Cd showed significant inhibition at 0.2 and 0.4 ppm with reduction in canola oil degradation of 43.63% and 50.1%, respectively, while Ag showed an effect at 0.6 ppm with reduction in degradation of 31.49%. The IC_{50} values of Hg, Cd and Ag were 0.39, 0.45 and 0.32 ppm, respectively. The strain can also withstand 10 mg/L acrylamide, 50 mg/L phenol and 0.5 % (v/v) diesel respectively. The present information regarding the effects of heavy metals and xenobiotics on canola oil degradation by this cold-tolerant bacterium can be useful to the real state for oil degradation in Antarctic environments exposed to multiple pollutants under low temperature.

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